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XXIV.—*On Fecundation in* Eudorina elegans *and* Cryptoglena.

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[With a Plate.]

For the last four years, just about the end of May and beginning of June, when the water in the tanks becomes very low, and is reduced to two or three pools, or a shower of rain makes such pools in those which are already dry, and when, in both instances, a development of *Euglena* has taken place, I have seized these opportunities, which are but of short duration, as the development soon becomes expended, to study, not only the *Euglenæ*, but *Eudorina*, *Gonium*, and *Chlamydococcus*, which all make their appearance at the same time, more or less together, and in such numbers as to give a deep-green tint to the water in which they are generated.

This year my attention has been more particularly directed to *Eudorina*; for in one pool I found it undergoing fecundation, which being similar to that described by Dr. F. Cohn in *Volvox globator**, an account of it will serve not only to confirm what that eminent Infusorialist has stated, but also to furnish a fresh instance of this process in another and closely allied organism.

Before, however, going to the fecundation, it is desirable that we should trace the development of *Eudorina* up to this point; but not having been able to recognize this organism in its simplest form, that is, as a solitary single cell, nor any stage of its *segmentation* prior to the third degree of duplicative subdivision (that is, into 16 cells, when the mother-coverings have dropped off), I must begin from this period.

At this time, which we will call the first stage, the *Eudorina*

* Comptes Rendus, t. xliii. p. 1054, Nov. 24, 1856.

consists of an ovoid green body, partially divided into the number of cells just mentioned, each of which is provided with a pair of cilia which project through a thin gelatinous envelope that surrounds the whole mass. It is now in its smallest size, about 5-5400ths of an inch long, that is, not more than the diameter of the *Chlamydococcus-cell*, fig. 9. Pl. VIII., and swims by means of its cilia, with the small end foremost, and with a rotatory motion on its longitudinal axis, as often from right to left as from left to right. An eye-spot is also present in each of the four anterior cells, but seldom visible in the rest at this period.

As the development progresses and the *Eudorina* increases in size, the division becomes complete, and each cell, in addition to the granular mucus and chlorophyll which line its interior, may now be seen to be provided internally with a spherical translucent utricle (which is the nucleus), an eye-spot situated peripherically and midway between the cilia and the opposite end of the cell, a contracting vesicle* at the base of the cilia, and the pair of cilia themselves. Each pair of cilia passes out through a single channel in the gelatinous cell or envelope, which has now become much thickened—and thus their movements are limited up to this point,—while a defined line internally marks the boundary of the original cell-wall, through which, of course, the cilia also pass (Pl. VIII. figs. 1, 2).

During the second stage, each of the cells again undergoes duplicative division (the nuclei having been doubled previously), and the whole organism becoming larger, they are separated from each other, and, being no longer subject to the compression which, with the lines of fissiparation tending towards the centre of the ellipse (see section, fig. 2), and their confined position, induced a more or less conical and polygonal shape, now become spherical and enclosed respectively within distinct transparent capsules (fig. 3). The *Eudorina* is now 30-5400ths of an inch long, and contains thirty-two green cells, which are evidently situated between two large, ovoid, colourless, transparent cells, one of which bounds a similarly-shaped cavity in the centre of the *Eudorina* (fig. 2 *c*), and the other is the original cell-wall (*a*), round which again is the newly secreted envelope (*b*),—while the green cells are further fixed in their respective positions by the passage of their cilia through the two latter, both original cell-wall and envelope (fig. 2 *h* and fig. 8 *c*). Thus we see that the *Eudorina* is derived from a simple (daughter-) cell, and that its green cells have resulted from a duplicative subdivision of the green matter which lined the cavity of this

* *Gonium* has two "contracting vesicles," unless, as in *Euglena*, one is a reservoir to the other.

cell*. Arrived at this state, which we shall presently see is that of maturity, we also observe that the posterior part of the envelope becomes crenulated, apparently from flaccidity (fig. 3 *g*).

After this, however, it again presents another phase, which may be called the third or last stage of development. Here *each cell* again undergoes a rapid duplicative subdivision into sixteen or thirty-two cells, which, in the group, assume a more or less oblong figure respectively; and thus the *Eudorina*'s length is increased to 50-5400ths of an inch. The internal structure now gradually breaks down before the external envelope, when for a short time the groups may be seen swimming about the cavity thus formed, till at last the envelope bursts and they become liberated. What becomes of them afterwards, I cannot state from observation; but the green cells having been greatly reduced in size by the latter subdivisions, it is probable that many of the groups, if they do not form new individuals, sooner or later become disintegrated, and the *Eudorina* thus eventually perishes †.

When, however, the process of impregnation takes place, the division stops at the second stage, that is, when the *Eudorina* consists of thirty-two cells of the largest kind, each of which is about 1-1866th of an inch in diameter within its capsule, which is therefore a little larger. The process is as follows:—

At a certain period after the second stage has become fully developed, the contents of the four anterior cells respectively present lines of duplicative subdivision which radiate from a point in the posterior part of the cell (and this distinguishes this subdivision from that (fig. 1) which took place in the original cell from which the *Eudorina* was derived, and that which takes place in the third or last stage of development just described, where the lines of fissiparation tend towards the centre of the ellipse or ovoid cell). These lines, which ultimately divide the green contents of the cell into sixty-four portions, where the division stops, necessarily entail (from their radiating from a point and terminating a little beyond the centre of the cell) a pyriform shape on the segments, from whose extremities a mass of cilia may be observed waving in the anterior part of the cell of the parent, while yet her own pair of cilia are in active motion, and her eye-spot still exists *in situ* on one side of her progeny,—thus showing that the latter may be almost fully

* It is assumed that the green contents get their cell-wall through in a plastic state, before the segmentation commences, as will be seen hereafter.

† For good illustrations of this stage, see Prof. Henfrey's description and plate of *Pandorina Morum*, Quart. Journ. Microscop. Soc. vol. iv. p. 49, 1856.

formed before the parent perishes (fig. 4 *a*). At length, however, this takes place, and the progeny, which we shall henceforth call 'spermatozoids,' separate from each other, and finding an exit, probably by rupture, through the effete parent-cell and her capsule, soon become dispersed throughout the space between the two large ovoid cells mentioned, where they thus freely come into contact with the capsules of the twenty-eight remaining or female cells (Pl. VIII. fig. 5).

The form of the spermatozoid now varies at every instant, from the activity of its movements and the almost semifluid state of its plasma; and therefore, if we had not seen it in the parent-cell (fig. 6), it would be very difficult to define what this form really is. Its changes in shape, however, are confined to elongation and contraction, like those of *Euglena viridis* (fig. 7), and not polymorphic, like those of *Amœba*; hence it is sometimes linear-fusiform or lunular, at others pyriform, short, or elongate. The centre of the body is tinged green by the presence of a little chlorophyll, while the extremities are colourless (fig. 7); the anterior one bears a pair of cilia, and there is an eye-spot a little in front of the middle of the body, also probably a nucleus. Thus we have a product widely different from the common cell of *Eudorina*. It is about 1-2700th of an inch long, and 1-10,800th of an inch broad.

Once in the space mentioned, the spermatozoids soon find their way among the female cells, to the capsules of which they apply themselves most vigorously and pertinaciously, flattening, elongating, and changing themselves into various forms as they glide over their surfaces, until they find a point of ingress, when they appear to slip in, and, coming in contact with the female cell, to sink into her substance as by amalgamation (fig. 5 *c*, *c*). I say "appear," because, the female cells as well as the spermatozoids being so small, so numerous, and so nearly grouped together, and there being no point like a micropyle that I could discover, and the *Eudorina* continually undergoing more or less rotation, I do not feel so certain of having seen the act of union take place as if there had been only a female cell present with a fixed point for the entrance of the spermatozoids, as in the resting-spore of *Œdogonium*. But the act itself does not require to be seen; for the constancy of this form of *Eudorina*, the way in which these little bodies are produced, their plastic nature, and their behaviour towards the female cells are quite sufficient to convince those who have given their attention practically to such subjects that they are spermatozoids, and that there can be no other object in their congregating about the female cells than impregnation. If this be not sufficient, their number may frequently be seen to diminish as they pass backward among the female cells,

when their disappearance can only be accounted for by their having become incorporated with the green cells. *Eudorina* in this stage also may frequently be seen with all the four anterior cells absent, and only a few spermatozoids left, most of which are motionless and adherent to the capsules,—indicating that the rest have disappeared in the way mentioned. Lastly, many *Eudorinæ* in this stage may be observed with not only the four anterior cells absent, but with hardly a single spermatozoid left,—indicating that the whole had passed into the female cells, or had become expended in the process of impregnation. I have never seen any spermatozoids in the central or axial cavity (fig. 2 *c*), nor do I think that there is a means of their escaping externally without rupture; so that their being confined to the space between the two ovoid cells of the *Eudorina*, where the green cells are situated, is another reason, if any more be needed, for considering them fecundating agents.

What changes take place in the *Eudorina* after this, I have not been able to discover. At the time, the female cells appear to become more opake by the incorporation of the spermatozoids, and the crenulated state of the posterior part of the envelope in this stage seems also to indicate an approach to disintegration. I have also observed that those *Eudorinæ* which are undergoing, or apparently have undergone impregnation, are less active than the rest,—that is, those in which the spermatozoids are scattered throughout the interspace mentioned and applying themselves to the capsules of the green cells, and those in which there are only a few spermatozoids left. But even if they did become disintegrated, the latter, when free, would so closely resemble those of *Chlamydococcus* (fig. 9), which was also abundantly present, that unless the *Eudorina* could be found undergoing impregnation by itself, or apart from this organism, there appears to me no chance of distinguishing the two, and therefore no other means of completing this part of its history. It is true that the impregnated cells may undergo some change in form similar to those of *Volvox globator* after impregnation*; but I think I should have seen this among the numbers which came under my observation, if it had been the case.

While undergoing impregnation, the female cells always contain from two to four nuclei, as if preparatory to the third stage of development (Pl. VIII. figs. 3 *h* and 4 *b*), into which they are sometimes actually seen passing, with the spermatozoids present and scattered among them; but the effect of impregnation generally seems to arrest this stage, and thus save the species from

* See Ehrenberg, tab. 4. figs. 2 & 3; also Busk, Quart. Journ. Microscop. Soc. vol. i. p. 38 and pl.; and Cohn, *l. c.*

that minute division which leads to the destructive termination of *Eudorina* already noticed.

Sometimes all the cells together undergo the spermatoid fissuration, when the *Eudorina* passes into *Pandorina Morum*, Ehr.; but in this case the development does not stop at the pyriform spermatozoids, but goes on to the development of thirty-two larger globular cells in each group, similar to those produced in the third stage of *Eudorina* above described, when they assume respectively a dome-shaped form, held together by a membrane which is fixed to the point in the posterior extremity of the cell from which the lines of fissiparation first radiated (fig. 8). As the groups, however, progress in development, this dome appears to become flatter, and, the *Eudorina* breaking up, as in the third stage, these groups, when liberated, finally appear to pass into the form of *Gonium*, when I think they perish, like the corresponding groups of the third stage. I did not observe this development (in which may be included some abnormal states, where one or two of the spermatic cells fail, and one or more of the female cells take on this mode of fissiparation irregularly) until the normal one of impregnation ceased to appear. Ehrenberg was wrong in giving the cells of *Pandorina* and *Eudorina* single cilia, as has before been stated; and partly wrong in leaving out the eye-spot; both of which, though disappearing ultimately, indicate the continued life of the parent-cell, as in the development of the spermatozoids, long after the formation of her progeny.

Thus the process of impregnation in *Eudorina* agrees closely with that described by Dr. F. Cohn in *Volvox globator*, in which organism I had seen some of the cells of the interior undergoing a spermatoid development exactly like that above described, and also that previously figured by Mr. Busk*, and alluded to by him as one of "microgonidia†;" and therefore the moment I perceived it in *Eudorina*, in connexion with Dr. Cohn's announcement, I felt convinced that the latter was right, and that I had before me *Eudorina* also undergoing a similar process of fecundation.

So much for the spermatoid development; let us now return to that of the *Eudorina* in totality, concerning which there is still an interesting question for our consideration, bearing on the early development of this organism, which I have already stated my inability to supply, viz. how does the sixteen-division of the cell in the third stage of development take place, so as to allow the cilia to become external? It will be remembered that this cell in the second stage, before it passes into the sixteen-division of the third stage, consists of its capsule or cell-wall and the

* *Loc. cit.* pl. 5. fig. 14. † *Id.* p. 45.

green contents; and it should also be remembered that, although these contents have now no other covering distinct from the protoplasm but the capsule, yet in all algal cells, whenever the green contents take on a new form, such as that of a spore or group of cells, a second more delicate covering is separated from them, for which I have heretofore used the term "protoplasmic sac*"; these two coverings, then, are the parental division of the mass, and become caducous as the rest takes on its new form and developes on *its* surface a cell-wall. Thus we get the sixteen cells separated from their capsule, &c., and surrounded by their proper cell-wall and the external envelope, which may be a still further thickening of the former, or a new secretion; but, be this as it may, the cilia are seen outside it. And at first it might be thought that they were formed before either the cell-wall or envelope, so as never to have been enclosed by either; but if this were the case, the cilia of the sixteen cells, which are added by duplicative division to the first stage of *Eudorina* to form the second stage, should be inside these coverings, or protrude through the original sixteen channels with the other sixteen pairs of cilia. However, neither is the case; for these sixteen cells have their channels respectively as well as the other sixteen cells, in which case they must have been made by the sixteen new cells themselves, unless the thirty-two division is formed before the pellicle, which subsequently forms the cell-wall, is supplied; and our first stage does not pass into the second stage, but both forms are produced at once and separately from the beginning,—a point which can only be determined by following the development of the *Eudorina* from the spore itself, and that, too, alone, since it is impossible to say whether the sixteen-division groups, when previously mixed up with all the other forms of *Eudorina*, are or are not derived direct from the spore, or from the third stage of development of this organism. That the sixteen-division or second stage may pass direct into a similar form to the third, that is, into a form of *Eudorina* consisting of sixteen groups of sixteen cells each, I have occasionally seen; but then this form has been globular (only 30-5400ths of an inch in diameter), and not ovoid, although the groups have possessed the latter form: perhaps this is the spore, and the sixteen groups the young *Eudorinæ*, if not a different species. Again, the robust individuals of the sixteen-division one would think to be direct from the spore, and to pass into the robust individuals of the second stage or thirty-two division,—while the puny, meagre individuals one would think to come from the third stage, and, as before conjectured, end in disintegration and death. But all this, as I have just stated,

* Annals, vol. i. 1858, p. 31, &c.

can only be determined by following the development of the spore from the commencement. One fact I might add, however, viz. that the robust forms about the size given in fig. 1 have the power of withdrawing their cilia and protruding them again; this happens when they are transferred, from the vessel in which they may be contained, to the slide for examination: many may just at this time be seen to be motionless, with the channels for the cilia empty; but gradually the cilia are protruded through them, and as gradually the *Eudorina* evinces increasing power of motion, until they are fully protruded, and it swims away.

Chlamydococcus undergoes the same kind of changes in development as *Eudorina*, from which it only differs in structure in being smaller, and globular instead of ovoid, in the absence of an external envelope, and in the cilia of the daughter-cells being *included* within the parent-cell; hence it also differs in being motionless, though the compartments of the daughter-cells are sufficiently large for them to turn round and move their cilia freely therein, which they are continually doing (figs. 9, 15). The primary cell of *Chlamydococcus*, like that of *Eudorina*, divides up into two, four, eight, or sixteen cells, and those of the eight- and sixteen-divisions again into groups of sixteen or thirty-two each (fig. 14), so as to resemble the third stage of *Eudorina*. Hence we may perhaps infer that its fecundating process is similar to that of *Eudorina*; but this remains to be discovered. *Chlamydococcus* has also a great tendency to stop at the two- and four-division, from which it may pass into the "still" or *Protococcus*-form, and, floating on the water in a kind of crust, present cells of all kinds of sizes undergoing "still" division. In all its multiplications, partial and entire, however, it generally maintains its primary or spherical form, and does not become ovoid or oblong, like the groups of *Eudorina*, the only exceptions being in the two- and four-division, where the green cells are sometimes ovate (probably from want of room in the parent capsule, fig. 11), as represented by Ehrenberg in *C. Pulvisculus**,—to which I should refer it, had he not also given an ovate form to the type-cell of this species†; nor can I refer it to *C. pluvialis*, for in all the changes I have yet seen it undergo, the red colour has not increased beyond the minute eye-spot, while this also disappears, and the cilia too, when this species passes into the "still" form (fig. 15). Here it undergoes the same kind of division that it does in the active state; but the parent-cell, instead of becoming distended by imbibition, remains closely attached to the daughter-cells, so as to give the group a

* Tab. 3. fig. 10.

† There is an ovate species, common in Bombay; but this has four cilia.

mulberry shape (figs. 16, 17). How long it remains in the "still" form I am ignorant; but having only seen it in the active state during the months of May, June and August, and throughout the rest of the year in the "still" one, I am inclined to think that it only comes into the active state during the summer months, and then for the purpose of fecundation.

Does not the disappearance of the eye-spot in the "still" form thus seem to point out its analogy with the bright colours, especially the red, presented by plants in their flowers during the season of fecundation, rather than with the eye of animals?

In several instances, also, where I have found this *Chlamydococcus* with *Eudorina*, they have been accompanied by long Closteriform cells. It was the case in that above mentioned, where the latter was undergoing impregnation. Some of these have an eye-spot, which, with the nature, arrangement, and general aspect of their internal contents, show that they belong to the class of organisms with which they are associated. Their cell-wall also is more or less plastic, or was so when they were assuming this spicular form; for many have one or more *diverticula* extending from them, some are bifid, and a few irregularly stellate. What they are, I know not; but Dr. Cohn has figured the same kind of cells, in company with *Sphæroplea annulina*, under impregnation*.

Trachelomonas, Ehr., also appears to me to undergo multiplication in a similar way to *Eudorina* and *Chlamydococcus*; for I have often seen the largest Trachelomonad of a pool divided up into a group of apparently sixteen cells within the lorica; and this may account for the myriads of three to four smaller sizes that are frequently found together in this way. The latter certainly appear in a green form first, that is, without the lorica, which gradually becomes supplied afterwards. Thus, impregnation also in the Trachelomonads may take place like that already mentioned in *Eudorina*, after the parent-cell has undergone division within the lorica. At first I thought that the first form of *Eudorina* arose in this way, and that when the division of the Trachelomonad arrived at sixteen, the lorica burst, and thus liberated a *Eudorina*; also that the cells into which the *Eudorina* ultimately divides formed the small Trachelomonads; but in the pools where I found the *Eudorina* undergoing impregnation there was not a single Trachelomonad, so that this theory does not hold good.

How *Euglena viridis* and the *Euglenæ* generally become impregnated, I have no conception. There is no doubt that *E. viridis* becomes distended with the cells which I have heretofore

* Ann. des Sc. Nat. t. v. pl. 12. fig. 3. Bot. 1856.

described, and thought to be ovules or embryonic cells*, and that during this time the chlorophyll passes into red grains and subsequently disappears, while the organism is secreting a capsule round itself, and its original cell-wall passes into a tough spherical ovisac, so to speak. But what becomes of this if it be the result of impregnation, or what the process of impregnation is like, or when it takes place, is for future discovery to determine. *E. viridis* does not become capsuled in this way, and is found floating on the water aggregated into layers one cell deep, or buried singly in the mud of tanks, after the capsulation has thus taken place.

It now only remains for me to state that my observations on *Eudorina* this year have been chiefly confined to two pools within two hundred yards of each other, one of which, viz. No. 1, is in a clean quarried excavation in the trap-rock, and the other, viz. No. 2, in an excavation in the soil. In both, which were previously dry, the rain fell on the 31st of May, so as to form small pools of water about two feet deep; and in both, on the 3rd of June, the water was tinged green with *Eudorina*, &c.

In No. 1, *Eudorina elegans, Chlamydococcus, Euglena viridis,* and an elliptical unicellular Alga abounded, to the exclusion of almost every other organism of the kind. The *Eudorina* was undergoing the process of fecundation, and the *Chlamydococcus* the transformations above described, respectively. On the 8th, the specimens of *Eudorina* under fecundation began to get scarce; and on the 13th the *Pandorina*-form made its appearance. By the 14th all organisms of the kind except the *Eudorina* and *Chlamydococcus* had disappeared, and the *Pandorina*-form was also rarely seen. The *Chlamydococcus* then began to float on the surface in the *Protococcus*-form above mentioned, and at length this sunk to the bottom, when, by the 19th of June, the remains of *Eudorina* and *Chlamydococcus* were but sparsely scattered through the water, which had now lost its green tint; indeed, it was evident that the development of all the green organisms had become exhausted. Up to this time there had been only a few more drops of rain; but the next morning the storms of the monsoon commenced.

In No. 2, *Eudorina elegans, Euglena viridis,* and *Trachelomonas* abounded, to the exclusion almost of every other organism of the kind; thus it contained no *Chlamydococcus,* while No. 1 contained no *Trachelomonas*. Corresponding changes took place in the *Eudorina* to those above described, with the exception of the impregnatory one.

Out of a dozen instances in which I have watched the deve-

* Annals, vol. xx. p. 36. pl. 1. fig. 16, 1857.

lopment of *Eudorina* during the past four years, I have thus only once met with it under fecundation.

As regards the effect of iodine (both by itself and assisted by sulphuric acid) upon *Eudorina*, I could never obtain a blue colour in the cells at any time; but it was distinctly visible in the transparent structures between them, perhaps in their capsules and the cellulose material which supported the rest of the organism. The contents of the cells always assumed a brown colour. The same was the case with the *Chlamydococcus*, though I see by my sketches that the solitary dividing still-cell, in the autumn of 1855, became blue throughout under the action of iodine and sulphuric acid.

Since the above was written, I have had the good fortune to meet with a *Cryptoglena* undergoing fecundation, of which the following is a description, both of the organism and the process:—

Cryptoglena lenticularis, nov. sp. Pl. VIII. figs. 18, 19.

Lorica lenticular, compressed, emarginate, uniformly and minutely granulated, transparent, colourless by transmitted, brownish by reflected light; margin of a deeper colour than the rest, probably from the proximity of the sides; presenting on the edge a notch anteriorly, one lip of which projects beyond and slightly overlaps the other, from which it is separated by an oblique fissure. Internal cell lenticular, compressed, one-fourth less in diameter than the lorica, lined with green chlorophyll and granular protoplasm, provided with a pair of cilia which pass out at the notch in the margin of the lorica, a single contracting vesicle at their base, a red eye-spot median and peripheral, and a nucleus. Lorica splitting into halves during fissiparation. Long diameter 1-1350th of an inch.

Found in most of the tanks and many of the wells in the island of Bombay. Active throughout the year.

Obs. This is the little *Thecamonadina* to which I have before alluded, as being associated with a species of *Œdogonium*, and which I wrongly conjectured to be a spore of this Alga*. It resembles *Cryptomonas lenticularis*, Ehr., in the compressed form and thickness of the lorica, while the bilabiate notch and oblique fissure in the margin ally it to *Euglena*, but not to *Trachelomonas* or *Lagenella*, which have a round aperture respectively for the exsertion of their cilium. It approaches *Chlamydococcus* in having two cilia, and in its mode of fissiparation, whilst it resembles *Schizochlamys*, Braun, in the splitting of the lorica.

* Annals, vol. i. 1858, p. 35.

Perhaps Ehrenberg's *Cryptomonas lenticularis*, being without eye-spot or cilia, is a "still" form of it; but it approaches nearest of all to his genus *Cryptoglena*.

I found this little Alga (for such in the end it must be considered) on the 2nd of July, in company with another heart-shaped *Cryptoglena*, which will be described presently, in great numbers in a little portion of shallow water connected by the rain with a pit into which the drainings of a buffalo-shed were received. With them were also *Euglena Acus*, *Eudorina elegans* (undergoing the process of fecundation above described), *Uvella Bodo*, Ehr., and here and there *Euglena viridis*; but the bulk of the organisms present consisted of the two first-mentioned; the rest, with the exception of *Euglena Acus*, were only now and then seen.

While examining some of this water, I was struck with the number of deciduous loricæ present, some of which were split into halves which were separated, while others only adhered together anteriorly, and presented a pair of cilia attached to their point of union (figs. 25–27); and on looking round for their origin, it was soon found that they belonged to the *Cryptoglena* above described, for the internal cell of that organism was in several instances seen escaping from them, not only singly, but after having undergone duplicative subdivision into two, four, eight, sixteen, thirty-two, or sixty-four gonidia (figs. 20–24). Moreover, it was observed that each of these groups came forth in a delicate cell (the protoplasmic sac*), which, by imbibition of water, became distended, in some instances, to two or three times the diameter of the lorica, and, thus assuming a globular form in the four-, eight- and sixteen-divisions, were undistinguishable from *Chlamydococcus* under similar forms (figs. 22, 23),—a point which still more nearly allies these organisms.

Still seeking for more of these varieties, it was observed that the first division, viz. that in which the internal cell came forth with only two gonidia, was invariably surrounded by a swarm of from ten to twenty much smaller gonidia (figs. 26, 27), which, on turning to the sixty-four division, were found to be identical, to all appearance, with the gonidia of this degree, of which there were numerous instances present, not only where the lorica was as yet unruptured, but where the internal cell had been liberated and the group were swarming within it, and where this cell had also become ruptured, and the gonidia were issuing one after another through the opening (fig. 25).

Now, the only organism present which was undergoing this subdivision being this *Cryptoglena*, and this, therefore, being the only

* See "Fecundation of *Œdogonium*," Annals, 3 ser. vol. i. p. 31, note.

one which could furnish the double spore-cell and smaller gonidia in large numbers, while in most instances the split lorica still adhered to the internal cell as a mark of identification, no doubt could be entertained that both the large and the small gonidia belonged to the same organism, that their coming together under such circumstances could only be for the purpose of fecundation, and that they therefore were the female cells and spermatozoids of this species.

I therefore watched the motions of the spermatozoids so situated, in two or three instances for more than half an hour together, during which time they dashed themselves against the cell, adhered to it, retreated from and advanced upon it, with unabated rapidity, but without penetrating it,—when, having little time left, comparatively, for such observations, I sought out group after group quickly, without watching it long at a time, until I had the good fortune to find one in which, after a moment's watching, one of the spermatozoids appeared within the cell, and, fixing itself to one of the spores or female cells, gradually became incorporated with it (fig. 26). This was sufficient to convince me of the fact I had anticipated; but it would have been more satisfactory to have seen it repeated, and probably I might have done so had the fecundating process in the colony been prolonged: but it only lasted three days after I first discovered it, and during that time I had little leisure to devote to the subject; for to be successful in researches of this kind requires uninterrupted observation for long periods together. However, had I not even seen the incorporation, no other interpretation could be given to the facts mentioned than that they were connected with the process of fecundation.

I have stated that the cell containing the two gonidia was the one invariably surrounded by the spermatozoids; but it was not the only one, for in two or three instances a few were found around and adhering to the inner cell of the four-division (fig. 21) after liberation, indicating that the gonidia of this division were also sought after by the spermatozoids, if even in the end they did not become reproductive. It was also observed that the two-division did not always come forth in one cell, but that sometimes this was also divided, so that each gonidium had its proper cell (fig. 27).

The form of the macrogonidia or female cells did not differ from the internal cell of the parent, except in being a little smaller,—while the microgonidium, which was not more than 1-7th of the diameter of the macrogonidium, and therefore very small, appeared, though equally green and provided with an eye-spot, to have only one cilium. I cannot help thinking, however, that, with a higher power, I might have seen two.

What changes took place in, and what became of, the macrogonidia after impregnation, I am unable to say; but it will be remembered that the only other organism present in equal number with the *Cryptoglena* above described, and to the exclusion almost of all other organisms, was one with a heart-shaped lorica. In this, however, no fissiparative changes like those above mentioned took place; but on the third day after the water containing them and the other *Cryptoglena* had been placed in basins, all other organisms disappeared but this, which had taken on the form of a resting-spore; that is to say, the cilia had shrunk up or had dropped off, the internal cell had become encapsuled within a thick cell externally and a thin one internally, within which, again, the chlorophyll had passed from a green into a light brick-red colour, and the granular protoplasm into a number of larger granules presenting a more or less uniform size and oleaginous refractive aspect (fig. 29). Whether these were the impregnated spores of *Cryptoglena lenticularis* or not, I have now no means of determining; for instead of collecting a few of them immediately and drying them for future experiment, this was deferred for a day or two, during which time some other organism (probably *Coleps*, the usual aggressor under these circumstances) ate them all up. All therefore that remains favourable to the inference is, that this spore, in its active state, was almost exclusively associated with *C. lenticularis*, and in equal number, and that it did not undergo fissiparation like this, but, on the contrary, passed into the form of a resting-spore. Against this, again, is the form of the lorica, and its having four cilia instead of two; hence, in case it should be a different species, it is desirable to give it a name and description, which may stand as follows:—

Cryptoglena cordiformis, nov. sp. Pl. VIII. fig. 28.

Lorica heart-shaped, round, transparent, emarginate anteriorly, round posteriorly. Internal cell globular, lined with chlorophyll and granular protoplasm, provided with four cilia, which issue through the notch in the lorica, an eye-spot median and peripheral, and one to three nuclei or utricles of a circular form. Swimming with its cilia forwards, and a rotating motion on its longitudinal axis. Length of lorica 1-933rd of an inch; diameter of internal cell 1-1350th of an inch.

Found only in one instance, in company with *Cryptoglena lenticularis*, when undergoing the process of fecundation above described.

Having thus established another mode of fecundation in an organism closely allied to the simplest of all vegetable forms,

viz. the unicellular Algæ, it becomes interesting to inquire how far the same process is likely to occur in the other families to which it is allied. I have stated above, that, from *Chlamydococcus* undergoing a similar duplicative subdivision to *Eudorina*, it is not improbable that it undergoes a similar mode of impregnation also; but in the fissiparation of *Cryptoglena lenticularis* we find such a resemblance to the compound groups of *Chlamydococcus*, that it now seems more likely that it should resemble that of *Cryptoglena lenticularis*. Again, I have stated that *Trachelomonas*, which is also a *Thecamonadina*, presents an appearance of fissiparation in its internal cell, which might enable it to undergo a fecundating process like *Eudorina*; but *Trachelomonas*, being loricated, is closely allied to *Cryptoglena lenticularis*, and therefore might rather be expected to undergo a fecundating process like the latter. The notch and oblique fissure also ally *Cryptoglena lenticularis* to the *Euglenæ*: but then the spiral-fibre coat of this family could not give way for the elimination of an internal cell with gonidia, like the lorica of the former; hence this fecundating process seems to throw no light on that of *Euglena*, which has yet to be discovered, and no doubt will be, sooner or later. But it is necessary to find out the time of the year when these processes are likely to take place, and where the organisms which are likely to be undergoing them make their appearance annually,—after which the water should be examined daily, immediately after the rain-falls; otherwise those which are required will be very likely to appear and go through the fecundating process (just as fast as some plants flower and seed) before their presence is even noticed. Hence, a place where there is rain occurring regularly only once a year is best fitted for these researches; and I feel convinced that, had I had better health and more leisure at the beginning of this "rains," I should have been able to have done much more in this subject than is above communicated.

August 7th.

P.S.—I have to-day seen the incorporation of the spermatozoid with the macrogonidium of *Cryptoglena lenticularis* take place several times, and once so satisfactorily that I am able to assert the fact without reservation. The former, after having fixed itself upon the latter, assumes a conical or peg-top shape, and thus gradually appears to squeeze itself into the macrogonidium. I have also ascertained that this spermatozoid has two cilia, as I had before supposed.

I would also notice here, that, in rating my micrometer, I have just found out that the 5600th and 1900th divisions should be 5400th and 1880th of an inch respectively. The reader is

therefore requested to make this correction in all my previous measurements where these divisions are mentioned, and to allow for this generally, indeed, in all my measurements.

[The necessary alterations have been made in the present paper.—Ed.]

EXPLANATION OF PLATE VIII.

N.B. All the figures in this Plate have been delineated as nearly after nature as the circumstances of the case would allow; and in order that their relative size may be seen, all, with the exception of figs. 6, 7 and 8, have been drawn on a scale of 1-12th to 1-5400th of an inch.

Fig. 1. *Eudorina elegans*, in its first stage of development, 9-5400ths of an inch long, composed of sixteen cells, surrounded by a gelatinous envelope, through which their cilia project: *a*, four anterior cells in which the eye-spot is visible; *b*, gelatinous envelope.

Fig. 2. Section of ditto, showing—*a*, gelatinous envelope; *b*, original cell-wall; *c*, internal cell enclosing axial cavity; *d*, interspace between original and internal cell-walls, in which the green cells are situated; *e*, nucleus; *f*, eye-spot; *g*, cilia; *h*, canals through gelatinous envelope for the passage of the cilia.

Fig. 3. *Eudorina elegans* in second stage of development, 30-5400ths of an inch long; composed of thirty-two green cells, each of which is 3-5400ths of an inch in diameter, exclusive of the capsule in which it is enclosed: *a*, *a*, *a*, *a*, spermatic cells, now undistinguishable from *b*, *b*, *b*, the female cells; *c*, shaded cell; *d*, nucleus; *e*, eye-spot; *f*, contracting vesicle; *g*, crenulated form of posterior extremity; *h*, nuclei in plurality.

In this, as well as in many other of these figures, the principal part of the cells and detail connected with them, as well as the shading, has been omitted, to save trouble, one or two complete ones being sufficient to show what the rest would be if all were properly filled in.

Fig. 4. Ditto, ditto, ditto, with the contents of the four anterior or spermatic cells transformed into tufts of spermatozoids respectively, —the presence of the eye-spot and movements of the cilia of the parent-cell indicating that she is still alive, though the cilia of her progeny are already waving in her interior: *c*, *c*, shaded female cells.

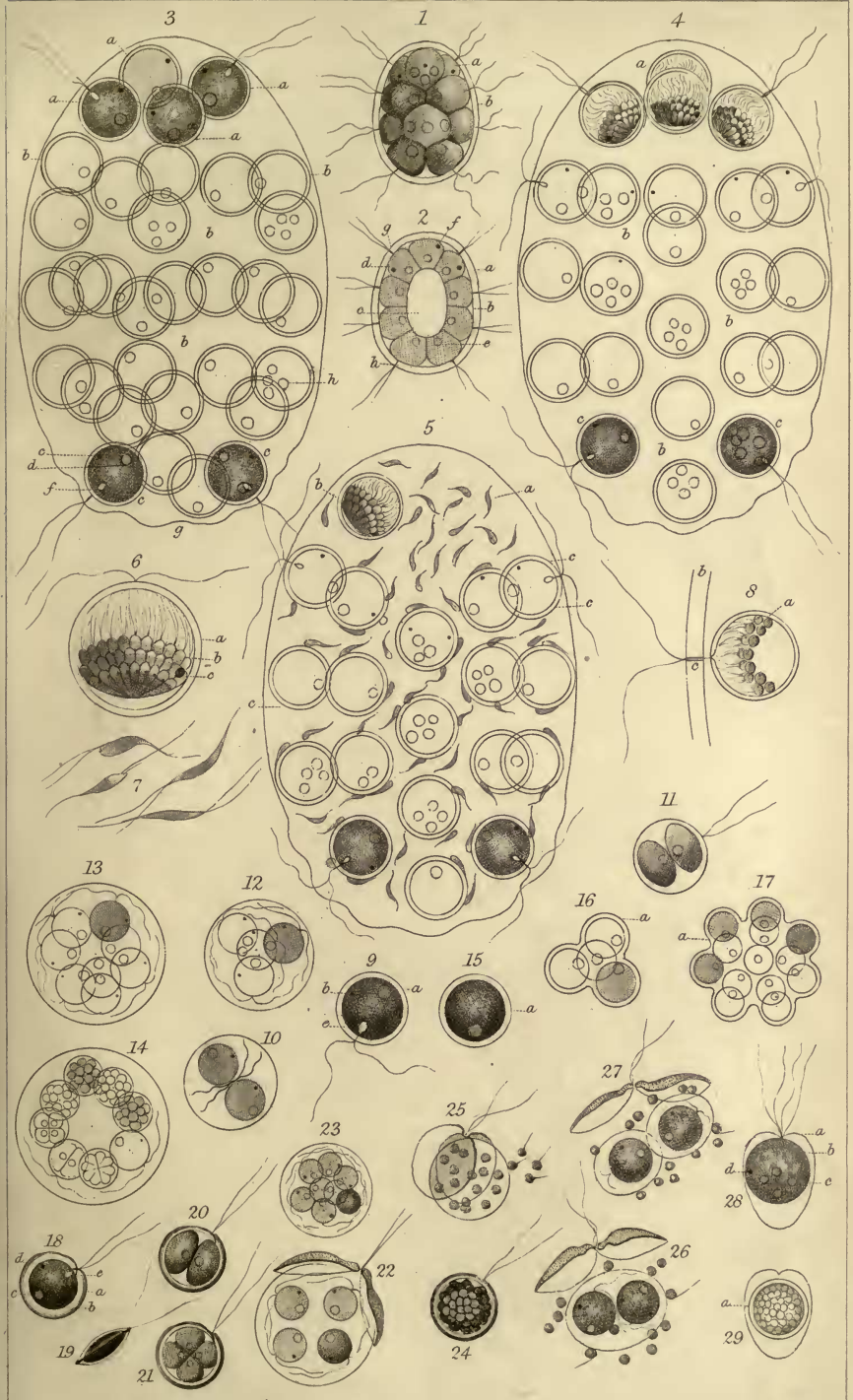
Fig. 5. Ditto, with three of the spermatic cells burst, and their spermatozoids scattered throughout the interspace between the outer and inner cell-walls, where they are vigorously applying themselves to the capsules of the green cells: *a*, spermatozoids apparently within the capsule; *b*, unruptured spermatic cell.

In this and in the foregoing figure, several of the green-cells are omitted, to avoid confusion.

Fig. 6. Single spermatic cell with tuft of spermatozoids more magnified, showing—*a*, capsule; *b*, mother-cell; *c*, eye-spot.

Fig. 7. Spermatozoids more magnified, to show the power they have of elongating themselves.

Fig. 8. A single cell of *Eudorina* in the second stage of development, but under the *Pandorina*-form, that is, where the contents of *all* the cells have undergone the spermatic mode of duplicative subdivision, but stop at sixteen or thirty-two, and pass into small cells of the ordinary form arranged in a tabular manner, like *Gonium*;



H.I.C. del.

J. Basire sc.